Effects of -1018G>A Polymorphism of HRH2 (rs2607474) on the Severity of Gastric Mucosal Atrophy

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Abstract

Background & Aim: Histamine plays important physiological roles in upper gastrointestinal tract and acts via the H2 receptor. A polymorphism -1018 G>A (rs2067474) was identified in an enhancer element of the HRH2 promoter. We attempted to clarify the associations of this polymorphism with the progression of gastric mucosal atrophy. Methods: Gastric mucosa samples were obtained from 398 subjects with no malignancies. The rs2067474 genotype was determined by PCR-SSCP method. The degree of gastritis was assessed in 366 subjects and serum pepsinogen (PG) I/II levels were measured in 108 subjects. The subjects with atrophy score ≥2 and metaplasia score ≥1 were classified into the severe atrophic gastritis group (SA group). Results: The -1018G>A minor allele frequencies in SA and non-SA groups were 8.02% and 13.3%, respectively (p=0.057). The -1018 GG homozygote had a significantly high risk for gastric mucosal atrophy (OR: 2.03, 95%CI: 1.03-4.01, p=0.042). In H. pylori positive subjects, GG homozygote was a more significant risk factor for gastric mucosal atrophy (OR: 2.32, 95%CI: 1.12-4.81, p=0.023). In addition, in the subjects older than 60 years, GG homozygote had also a significant risk for gastric mucosal atrophy (OR: 2.63, 95%CI: 1.15-6.00, p=0.022). In -1018 GG homozygote, PG I/II ratio was significantly lower in H. pylori positive than negative subjects and was significantly decreased with age (p=0.0032 by ANOVA), whereas it was not in the A carrier. Conclusions: Our results suggest that HRH2 -1018 GG homozygote is a risk factor for the severity of gastric mucosal atrophy under the influence of H. pylori infection, especially in older subjects.

Key words

Histamine H2 receptor – genetic polymorphism – chronic gastritis – Helicobacter pylori infection.

Introduction

The stomach is exceedingly rich in the following peptide hormone- or active amine-producing cells: enterochromaffin-like (ECL) cells (histamine), D cells (somatostatin), EC cells (serotonin), and G cells (gastrin) [1]. Histamine, one of the active amine released in response to a variety of physiological stimuli, is widely distributed in the gastrointestinal tracts including the stomach and it is involved in the pathogenesis of gastro-duodenal ulceration and gastric inflammation [2]. Histamine modulates a variety of functions by interacting with specific receptors on the target cells, H1, H2 and H3 receptors [3]. In the stomach, H2 receptors, although widely distributed in body tissues, seem to have a central role only in the regulation of acid secretion, as confirmed by the widespread use of H2 receptor blockers in the therapy of acid-related disorders [4, 5]. Recently, the Helicobacter pylori (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 [6, 7]. Evidence was also provided that increased gastric histamine contributes to the inflammatory changes and tissue damage associated with chronic H. pylori infection of the gastric mucosa [8, 9]. Thus, intragastric histamine plays an important role on the gastric inflammation acting via H2 receptor, although H. pylori infection is one of the major contributing factors to the development of gastro-duodenal inflammation [10].

On the other hand, the association between genetic polymorphisms of histamine receptor genes and the susceptibility to schizophrenia, and its clinical response to clozapine treatment has been studied [11]. This investigation of the H2 receptor gene polymorphisms reveals the association between genotype at the H2-1018-G/A locus (rs2607474) and clinical response to clozapine treatment, although upon correction for multiple testing this difference
is no longer significant. More recently, it has been reported that no significant relationship exists between -1018G>A polymorphism of histamine H2 receptor gene and the susceptibility to Parkinson's disease [12]. Histamine H2 receptor is encoded by \textit{HHR2}, located in chromosome 5q35.2, and rs2607474 is located in an enhancer element of the \textit{HHR2} promoter [11]. It is likely that the \textit{HHR2} variant located in the promoter may induce changes in the expression of receptors. There has been no report whether -1018G>A polymorphism (rs2607474) affect the development of and susceptibility to gastrointestinal disorders or not, although the investigators in the gastroenterological field have been interested in histamine H2 receptors for a long time.

This study is aimed at testing the hypothesis that genetic alteration in \textit{HHR2} may cause an increased risk for the severity of gastric mucosal atrophy. In an attempt to identify factors related to increased risk, we mapped common polymorphism in the \textit{HHR2} in the subjects with or without gastric mucosal atrophy as a premalignant condition.

\section*{Methods}

\subsection*{Clinical samples}

The study population comprised 398 subjects with no neoplastic lesions, who were enrolled at the Endoscopy Center of Fujita Health University Hospital or Kanazawa Medical University between 2006 and 2011. Patients with severe systemic diseases were excluded. All subjects underwent upper gastrointestinal endoscopy and, in some of them, biopsy specimens were taken from the antral mucosa. Parts of each specimen were fixed in 10\% buffered formalin and embedded in paraffin, while the other part was immediately frozen and stored at -80°C. Later, genomic DNA was isolated from the frozen specimens by digestion using proteinases K. In one part of them, genomic DNA was also isolated from peripheral blood using FlexiGene DNA Kit (QIAGEN GmbH, Hilden, Germany).

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

The Ethics Committees of Fujita Health University and Kanazawa Medical University approved the protocol, and written informed consent was obtained from all of the participating subjects.

\subsection*{Genotyping of polymorphisms}

Sample stocked DNAs isolated from biopsy specimens or peripheral blood were used. Polymorphism was genotyped by PCR-SSCP method as reported previously [13, 14]. To detect -1018G>A genotype, using the primer pair (forward: 5'-acctgacccttttctgaaaaagtttgtc-3', and reverse: 5'-ctactcctctgaagtgctgagaaccat-3'), PCR was carried out in a volume of 20 mL containing 0.1 mg of genomic DNA. The DNA was denatured at 95°C for 3 minutes, followed by 35 cycles at 96°C for 15 seconds, 60°C for 30 seconds, and 72°C for 30 seconds, with the final extension at 72°C for 5 minutes. Thereafter, 2 mL of the PCR product was denatured with 10 mL of formamide (Sigma-Aldrich Co., St. Louis, USA) at 95°C for 5 minutes. SSCP was carried out at 6°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).

\subsection*{Histological evaluation}

In 366 of 398 subjects, the severity of chronic gastritis was classified according to the updated Sydney system [15] by a pathologist who had no access to any clinical information. According to the severity of gastric mucosal atrophy, the subjects were divided into the following two groups: the non-severe atrophy (non-SA) group (atrophy score \leq 1 and metaplasia score=0), and the severe atrophy (SA) group (atrophy score \geq 2 or metaplasia score \geq 1).

\subsection*{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 108 of 398 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 [16, 17].

\subsection*{Statistical analysis}

The age was expressed as mean ± SD. Mean ages between two groups were compared by Student’s t-test. The ratios of \textit{H. pylori} infection status and male/female were 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 genotype association with the disease was assessed by calculating the odds ratio (OR) and 95\% confidence intervals (CI) by logistic regression analysis using dominant model (GG vs. GA+AA). Adjusted ORs were calculated with the use of a regression analysis after adjustment for age, gender and \textit{H. pylori} infection status. The PG I/II ratio between two groups was compared by Mann Whitney U-test. The relationship between age and PG I/II ratio was assessed by ANOVA. For all analyses, the level of significance was set at p<0.05.

\section*{Results}

\subsection*{Characteristics of subjects and the frequencies of genotypes}

Single strand DNAs of -1018G>A genotype were clearly separated by SSCP (Fig. 1). The distribution of -1018G>A genotype in all 398 subjects was 306GG, 88GA and 4AA (Table I). It was in the Hardy-Weinberg equilibrium (p=0.77).

The characteristics of subjects in this study were summarized in Table I. With respect to gastric mucosal atrophy, 260 and 106 of the subjects were classified into the non-SA and SA groups, respectively. The mean age and male/female ratio of the SA group were significantly higher.
HRH2 polymorphism and atrophic gastritis

The association between HRH2 gene polymorphism and atrophic gastritis was investigated. The H. pylori positive ratio of the SA group was significantly higher than that of the non-SA group. The distribution of -1018G>A genotype in the SA group was 89GG, 17GA, and 0AA, whereas the distribution in the non-SA group was 194GG, 63GA, and 3AA. The -1018G>A minor allele frequencies in the SA and non-SA groups were 8.02% and 13.3%, respectively (p=0.057). In addition, the frequency of -1018 GG homozygote tended to be significantly different between the SA and non-SA groups (p=0.055).

Association between HRH2 gene polymorphism and gastric mucosal atrophy

Overall, -1018GG homozygote had a significantly increased risk for gastric mucosal atrophy by logistic regression analysis after adjustment for age, gender and H. pylori infection status (OR, 2.32; 95%CI, 1.123-4.81; p=0.023), whereas no significant deviation of genotype distribution was found in H. pylori negative subjects (Table III). In addition, -1018 GG homozygote had also an increased risk in the subjects older than 60 years (OR, 2.63; 95%CI, 1.15-6.00; p=0.022, Table IV).

Serum pepsinogen levels in -1018 GG homozygote and A carrier

In 108 subjects with measured serum PG levels, 65 were H. pylori positive and 43 were negative. The distribution of genotype in H. pylori positive was 53GG, 11GA and 1AA, whereas the distribution in H. pylori negative was 30GG, 12GA and 1AA. In -1018GG homozygote, PG I/II ratio was significantly lower in H. pylori positive than negative subjects (p<0.0001), whereas there was no significant difference among two groups in the A carrier (Fig. 2). In addition, serum PG I/II ratio was significantly decreased with age in GG homozygote (p=0.0032 by ANOVA and |R|=0.32), whereas there was no significant correlation between age and PG I/II ratio in the A carrier (p=0.56 and |R|=0.12).

Table I. Characteristics of the subjects and frequency of genotypes

<table>
<thead>
<tr>
<th>Total</th>
<th>non-SA group</th>
<th>SA group</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>398</td>
<td>260</td>
<td>106</td>
</tr>
<tr>
<td>Mean age±SD</td>
<td>59.8 ± 13.3</td>
<td>58.1 ± 13.7</td>
<td>65.3 ± 10.4</td>
</tr>
<tr>
<td>Male : female</td>
<td>234 : 164</td>
<td>139 : 121</td>
<td>77 : 29</td>
</tr>
<tr>
<td>H. pylori positive rate</td>
<td>63.6 %</td>
<td>51.1 %</td>
<td>95.2 %</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>HRH2 -1018G&gt;A</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>GG</td>
<td>306</td>
</tr>
<tr>
<td>GA</td>
<td>88</td>
</tr>
<tr>
<td>AA</td>
<td>4</td>
</tr>
</tbody>
</table>

-1018A frequency | 12.1% | 13.3% | 8.02% | 0.057b |

*: non-SA group vs. SA group; a: the frequency of GG homozygote; b: -1018G>A minor allele frequency

Table II. Association between -1018G>A polymorphism and gastric mucosal atrophy*

<table>
<thead>
<tr>
<th>Genotype</th>
<th>OR (95%CI.)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HRH2-1018G&gt;A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>GA</td>
<td>AA</td>
</tr>
<tr>
<td>non-SA group</td>
<td>95</td>
<td>31</td>
</tr>
<tr>
<td>SA group</td>
<td>32</td>
<td>2</td>
</tr>
</tbody>
</table>

H. pylori positive subjects

<table>
<thead>
<tr>
<th>Genotype</th>
<th>OR (95%CI.)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HRH2-1018G&gt;A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>GA</td>
<td>AA</td>
</tr>
<tr>
<td>non-SA group</td>
<td>99</td>
<td>32</td>
</tr>
<tr>
<td>SA group</td>
<td>86</td>
<td>14</td>
</tr>
</tbody>
</table>

*: logistic regression analysis after adjustment for age and gender

Table III. The risk of -1018 GG homozygote for the gastric mucosal atrophy*

<table>
<thead>
<tr>
<th>Younger than 60 years</th>
<th>Older than 60 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td>OR (95%CI.)</td>
</tr>
<tr>
<td>HRH2-1018G&gt;A</td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>GA</td>
</tr>
<tr>
<td>non-SA group</td>
<td>102</td>
</tr>
<tr>
<td>SA group</td>
<td>19</td>
</tr>
</tbody>
</table>

*: logistic regression analysis after adjustment for gender and H. pylori infection status
In this study, we investigated the association between \(-1018G>A\) polymorphism of \(HRH2\), encoding histamine H2 receptor, the genotype of which is in the Hardy-Weinberg equilibrium, and the severity of gastric mucosal atrophy. The subjects were patients with upper gastroesophageal symptoms who had undergone gastroduodenal endoscopy. Among the participants, 106/366 had severe gastric mucosal atrophy and the overall \(H. pylori\) positive ratio of these subjects was comparatively high (63.6%). On the basis of these facts, overall \(HRH2\)-1018 A allele frequency was 12.1%. A previous study reported that the A allele frequency of \(HRH2\)-1018 was lower in the Caucasians than our subjects [11, 12]. In addition, the frequency in our subjects was slightly higher than that reported in Hap-Map JPT and lower than that reported in Japanese by Ito et al [18]. Another limitation of this study was that the effect of type II error cannot be excluded in relatively small sample sizes.

It has been well known that \(H. pylori\) infection has a major role on the progression of gastric mucosal atrophy. Infection with \(H. pylori\) first induces chronic superficial gastritis, which can progress to chronic atrophic gastritis, intestinal metaplasia, and dysplasia that leads toward gastric carcinoma [19]. Indeed, 100 from 105 subjects in SA group were \(H. pylori\) positive in our study. However, there are marked inter-individual differences in the extent of the inflammation among persons with \(H. pylori\) infection, so clinical consequences only develop in a subgroup. 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 the infection and precedes the development of atrophic gastritis. 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 [20, 21]. On the other hand, 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 [22-24]. Therefore, hypergastrinemia and/or insufficient acid secretion seem to promote the gastric mucosal atrophy under the influence of \(H. pylori\) infection. However, Takaishi et al have reported that the gastrin-histamine axis contributes to the development of gastric atrophy and neoplasia in the mouse model [25]. In contrast to the effects of hypergastrinemia seen in gastrin transgenic mice, long-term treatment of rats and mice with lortidine, one of potent histamine H2 receptor antagonists, did not result in the loss of parietal cells but instead appeared to result in increased parietal cell mass [26]. In addition, histidine decarboxylase knockout (HDC-/-) mice kept on a low-histamine diet showed an expanded parietal cell pool despite exhibiting marked hypergastrinemia [27]. 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. Therefore, we hypothesized that \(HRH2\) polymorphism may affect the gastric mucosal atrophy.

Fig 2. The serum PG I/II ratio and \(HRH2\)-1018G>A genotype In \(-1018\) GG homozygote, PG I/II ratio was significantly lower in \(H. pylori\) positive than negative subjects. In addition, serum PG I/II ratio significantly decreased with age in GG homozygote.
There have been few reports that demonstrate the influence of polymorphisms of HRH2 in the risk for human disorders. Most studies reveal no association between HRH2 -1018G>A polymorphism and schizophrenia or Parkinson’s disease [11, 12, 18]. On the other hand, there is not one report regarding the association between HRH2 -1018G>A polymorphism and gastric disorders. Our results provided the first evidence that HRH2 -1018G>A polymorphism, with function still unclear, was significantly associated with atrophic gastritis. Results from our study suggest that HRH2 -1018 GG homozygote may be significantly associated with a risk for the severity of gastric mucosal atrophy, especially in H. pylori infected and comparative elderly subjects. In addition, PG I/II ratio was significantly lower in H. pylori infected than uninfected subjects and significantly decreased with age only in -1018 GG homozygote. These findings suggested that age-related gastric mucosal atrophy gradually and rapidly progressed in the GG homozygote rather than the A carrier.

Conclusion

The current findings indicate that the HRH2 -1018 G>A polymorphism (rs2607474) influences the risk for the severity of gastric mucosal atrophy under the influence of H. pylori infection in Japanese subjects.

Conflicts of interest

Nothing to declare

References