Histamine $N$-methyltransferase 939A $>$ G polymorphism affects mRNA stability in patients with acetylsalicylic acid-intolerant chronic urticaria

**Background:** Histamine plays an important role in allergic inflammation. Histamine levels are regulated by histamine $N$-methyltransferase (HNMT).

**Objective:** To investigate the functional variability of HNMT gene in relation to genetic polymorphisms in patients with aspirin intolerant chronic urticaria (AICU).

**Methods:** Two single-nucleotide polymorphisms of the HNMT gene (314C $>$ T, 939A $>$ G) were genotyped in chronic urticaria patients. The functional variability of 3'-untranslated region polymorphism (3'-UTR) was assessed using the pEGFP-HNMT 3'-UTR reporter construct to examine mRNA stability and fluorescence-tagged protein expression. The HNMT enzymatic activities related to the 939A $>$ G polymorphism were examined both in the human mast cells (HMC-1) transfected with the pHNMT CDS-3'-UTR construct and in the patients' red blood cells (RBCs). Histamine release from the basophils of AICU patients was examined.

**Results:** The 939A $>$ G polymorphism was significantly associated with the AICU phenotype, while no association was found with the 314C $>$ T polymorphism. An *in vitro* functional study using HMC-1 cells demonstrated that the 939A allele gave lower levels of HNMT mRNA stability, HNMT protein expression, and HNMT enzymatic activity and higher histamine release than the 939G allele. The *in vivo* functional study demonstrated that the AICU patients with the 939A allele had lower HNMT activity in RBC lysates and higher histamine release from their basophils.

**Conclusion:** The HNMT 939A $>$ G polymorphism lowers HNMT enzymatic activity by decreasing HNMT mRNA stability, which leads to an increase in the histamine level and contributes to the development of AICU.

Department of Allergy & Rheumatology, Ajou University School of Medicine, Suwon, Korea

Key words: chronic urticaria patients with aspirin hypersensitivity; genetic polymorphism; histamine $N$-methyltransferase; mRNA stability.

Dr Hae-Sim Park
Department of Allergy and Rheumatology
Ajou University School of Medicine
San-5 Wonecheondong
Youngtonggu
Suwon 442-721
Korea

S.-H. Kim and Y.-M. Kang contributed equally to this work as first authors.

Accepted for publication 19 April 2008

Histamine is a bioactive chemical mediator that is released from basophils or mast cells after the binding of an allergen to IgE-occupied high-affinity IgE receptors (1, 2). Histamine plays various roles in allergic reactions in humans; it functions as a bronchoconstrictor in the lungs, a key mediator of wheal formation on the skin (3, 4), a controller of gastric acid secretion, and a neurotransmitter in the brain (5, 6). The cellular levels of histamine are regulated by the histamine synthesis enzyme $\ell$-histidine decarboxylase (HDC), and two metabolizing enzymes, histamine $N$-methyltransferase (HNMT) and diamine oxidase (DAO) (7–9). After the formation of histamine from $\ell$-histidine, histamine is quickly metabolized to $N$-methylhistamine by HNMT.

The role of histamine in the pathophysiology of chronic urticaria has been investigated extensively, as histamine released from cutaneous mast cells directly provokes wheal formation, flares, and itching in the early phase (10) and the clinical symptoms can be controlled by antihistamine (11). Chronic urticaria has been defined as the presence of daily itchy wheals that persist for more than 6 weeks, and the prevalence of chronic urticaria is approximately 3% in the general population (12, 13). About 20–30% of patients with chronic urticaria experience exacerbation of their condition after taking aspirin or other nonsteroidal anti-inflammatory drugs (14). In the Korean population, aspirin hypersensitivity

Abbreviations: 3'-UTR, 3'-untranslated region; AICU, chronic urticaria patients with aspirin hypersensitivity; aspirin-intolerant chronic urticaria; ASA, acetylsalicylic acid; ATCU, chronic urticaria patients without aspirin hypersensitivity, aspirin-tolerant chronic urticaria; CDS, coding sequence; DAO, diamine oxidase; HDC, $\ell$-histidine decarboxylase; HNMT, histamine $N$-methyltransferase; NC, normal control subjects; SNP, single-nucleotide polymorphism.
confirmed by oral aspirin provocation testing has been observed in 35.7% of patients with chronic urticaria (15). Although the key role of histamine in chronic urticaria is well-known, there are no published data on the functional contribution of HNMT to the pathogenesis of chronic urticaria. In this study, we hypothesized that functional variants of the HNMT gene because of genetic polymorphisms might be important in the regulation of histamine during allergic inflammation, especially in patients with chronic urticaria.

Two common genetic polymorphisms of the HNMT gene have been reported: 314C>T (T105I), which results in a change of amin acid 105 from threonine to isoleucine, and 939A>G, which is located in the 3'-UTR. A nonsynonymous 314C>T polymorphism was first reported in an American population, and its functional significance has been characterized; the 314T allele appears to be associated with a decrease in the enzymatic activity of HNMT (16). Therefore, the presence of the 314T allele may result in reduced histamine metabolism (17). Another previous study has reported that the frequency of the 314T allele in Caucasian asthmatic patients is significantly higher than in normal controls (NC), which indicates a significant decrease in HNMT enzymatic activity among asthmatics (18). However, a similar study carried out in an Indian population failed to find any association between the C314T polymorphism and atopic asthma (19). The 939A>G polymorphism, which was first identified in healthy subjects from a Chinese population (20), may affect the enzymatic activity of HNMT because of its location in the regulatory 3'-UTR. To the best of our knowledge, no previous study has provided in vitro or in vivo evidence that the 939A>G polymorphism affects the stability of HNMT mRNA, its enzyme activity, or histamine release in ASA (acetylsalicylic acid)-intolerant chronic urticaria (AICU) patients.

This study was undertaken to investigate whether two common genetic polymorphisms of the HNMT gene are associated with chronic urticaria, especially AICU, by examining the genotype distributions in three groups: AICU, ASA-tolerant chronic urticaria (ATCU), and NC. Moreover, we performed in vitro and in vivo functional studies of the HNMT 939A>G polymorphisms, to clarify the underlying molecular mechanisms.

Materials and methods

Subjects

Study subjects were enrolled at Ajou University Hospital in Suwon, Korea, and were classified into three groups: AICU, ATCU, and NC. The AICU phenotype was defined by oral provocation with 500 mg aspirin (Rhonal; KunWha Pharmaceutical Co., Seoul, Korea), as described previously (21, 22). All of the subjects who participated in this study were ethnic Koreans. The study protocol was approved by the institutional review board of Ajou University Hospital, Suwon, Korea, and all subjects were fully aware of the scope of this study, and had signed an informed consent form. Skin prick tests were performed with 12 common aerollergens (Bencard Co., West Sussex, UK). Based on the results of the skin prick tests, atopy was defined as one or more positive reactions to common inhalant allergens. Total IgE concentrations were measured using the UniCAP system (Pharmacia Diagnostics, Uppsala, Sweden), according to the manufacturer’s instructions. Anti-thyroglobulin (anti-Tg RIA) and anti-microsomal (anti-TPO RIA) antibodies in serum samples were detected using a radioimmunoassay (B R A H M S Aktiengesellschaft, Hennigsdorf, Germany). Serum antinuclear antibody was detected by indirect immunofluorescence (Fluoro HEPANA test; Medical & Biological Laboratories Co. Ltd., Nagoya, Japan).

Urticaria severity scores

We modified urticaria severity scores by using a previously reported method (23, 24). Urticaria severity score was estimated according to the number, size, duration, and distribution of wheals present at the assessment time and scored as follows: the number of wheals was scored 0, no wheals; 1, 1–10 wheals; 2, 11–20 wheals; 3 > 20 wheals. The size of wheals was scored 0, no wheals; 1, < 1 cm (in mean diameter of two directions); 2, 1–3 cm; 3, > 3 cm. The duration of wheals was scored by 0, no wheals; 1, < 4 h; 2, 4–12 h; 3, > 12 h. The distribution of wheals was scored by percentage of body surface area; 0, no wheals; 1, > 25%; 2, 25–50%; 3, > 50%. Urticaria severity score was expressed maximum of 12, which includes score of all four parameters (number, size, duration, and distribution). Subjective symptoms scoring was checked by visual analog scale, 0–10 scores.

SNP genotyping

Genomic DNA was extracted from 0.3 ml of whole blood with the Puregene DNA purification kit (Genta, Minneapolis, MN, USA) and frozen at –70°C. Single-nucleotide polymorphism (SNP) genotyping of two common HNMT polymorphisms (314C>T and 939A>G) was performed using the SNaPshot primer extension kit (Applied Biosystems, Foster City, CA, USA). The sequences of the amplifying and extension primers were as follows: for 314C>T, forward 5'-ATGTTGTGTGCACCTTCTCTC-3', reverse 5'-ATGTTGTGTGCACCTTCTCTC-3'; for 939A>G, forward 5'-CTCCTTATGCAAGCA-3', and extension 5'-CTCGTGATTTT-3', and for 939A>G, forward 5'-AGCCATACTATTCAATCGAAAATGATA-3', reverse 5'-TTATCTACATTGATGATGTTTTC-3', and extension 5'-TGAGTTGATATTATACTTTGAAA-3'.

Preparation of the pEGFP-HNMT 3'-UTR construct and mRNA stability assay

A 535-bp fragment (nt 880–1415) of the HNMT 3'-UTR of the human HNMT gene (NM_006895) was prepared by PCR amplification using either the 939A homoyzogte or 939G homoyzogte human cDNA as template. The forward primer sequence was 5'-CAAGCCGCCGCTATCAATCACAAAATGATATTC-3' (boldface characters indicate a NotI site) and the reverse primer sequence was 5'-CAACCTCGAGTGCACACATGATGTTAAAAAGTATA-3' (boldface characters indicate an XhoI site). The amplification conditions were initial denaturation for 5 min at 95°C, followed by 31 cycles of 30 s at 95°C, 30 s at 60°C, and 45 s at 72°C, with a final 7 min of extension at 72°C. Each of the PCR products were separately subcloned into the NotI and XhoI sites of pEGFP, which was previously constructed by ligation of the pcDNA3 vector (Invitrogen, Carlsbad, CA, USA) with the gene for enhanced green fluorescent protein (EGFP) upstream of the multicloning site.
Human mast cells (HMC-1) were cultured in IMDM (Invitrogen) medium that was supplemented with 10% FBS (Invitrogen), 100 U/ml penicillin, 25 mM HEPES, and 100 mg/ml streptomycin in 5% CO2 at 37°C. HMC-1 cells were transiently transfected with the EGFP reporter plasmid (pEGFP-HNMT 3'-UTR) using the MP-100 microporator (Digital Biotech, Seoul, Korea), according to the manufacturer's instructions. Briefly, HMC-1 cells were seeded at 1 × 106 per well in six-well plates in 2 ml of IMDM medium with 10% FBS. The EGFP reporter plasmid (2 µg of pEGFP-HNMT 3'-UTR) and 0.5 µg of a β-galactosidase-expressing control plasmid were transfected into HMC-1 cells and incubated for an additional 18 h. After the addition of 10 µg/ml actinomycin D (Sigma, St Louis, MO, USA), the HMC-1 cells were collected at different time-points (0, 0.5, 1, 2, 4, and 8 h).

From the HMC-1 cells collected at different time-points, total RNA samples were extracted using TRIZol Reagent (Invitrogen). Total RNA (2 µg) was reverse-transcribed using oligo(dT) primer and MMLV reverse transcriptase (Promega, Madison, WI, USA). The stability of the mRNA for each SNP was examined by real-time PCR in the ABI PRISM 7500HT thermocycler (Applied Biosystems, Foster City, CA, USA). The PCR reactions consisted of cDNA product (20–200 ng), 250 mM primer, and 12.5 µl SYBR Green PCR Master Mix (SYBR Green dye, AmpliTaq Gold, DNA polymerase, dNTP mix, and optimized buffer components; Applied Biosystems) in a total volume of 25 µl. To quantify EGFP reporter gene expression, two different primers were used for real-time PCR as follows: forward, 5'-ATGGAGAGTGTAGAAGAAATG-3' (for EGFP); reverse, 5'-GCTACATCTCATATCCAAACAG-3' (for HNMT). The following pair of primers was used for real-time PCR of the β-galactosidase gene as an internal control: sense, 5'-TATAGTTAACCCATTTAACCAAC-3' (for HNMT). The PCR products were collected after two washes with PBS (pH 7.2). The HNMT enzymatic activities in the red blood cells (RBCs) of AICU patients and NC subjects were examined using the method of Pazmino and Weinhilboum (25). Briefly, 5-ml samples of blood were obtained from each subject and centrifuged at 800 g for 1 min at 4°C. The pellet was added to 3 ml of 0.9% NaCl and the suspension was recentrifuged at 800 g for 1 min at 4°C. The supernatant and buffy coat layers were then removed. The RBC pellet was washed twice with PBS and centrifuged at 800 g for 1 min at 4°C. The RBC pellet was resuspended in 2 ml of PBS and 8 ml of ice-cold water. The activity of HNMT was measured by the method described above.

Histamine release from the peripheral blood leukocytes of the AICU patients was examined using the method of Bae et al. (26). For the histamine release assay, 10 µg/ml of anti-human goat IgE antibody (KPL, Gaithersburg, MD, USA), which was determined as the optimal concentration in a preliminary experiment, was used. Histamine concentrations were measured using the Histamine EIA kit (Immunotech) according to the manufacturer's instructions. The absorbance at 405 nm of each sample was measured in the Synergy HT microplate reader (BioTek Instruments).

**Association of HNMT genetic polymorphism with AICU**

The HNMT enzymatic activities in the red blood cells (RBCs) of AICU patients and NC subjects were examined using the method of Pazmino and Weinhilboum (25). Briefly, 5-ml samples of blood were obtained from each subject and centrifuged at 800 g for 1 min at 4°C. The pellet was added to 3 ml of 0.9% NaCl and the suspension was recentrifuged at 800 g for 1 min at 4°C. The supernatant and buffy coat layers were then removed. The RBC pellet was washed twice with PBS and centrifuged at 800 g for 1 min at 4°C. The RBC pellet was resuspended in 2 ml of PBS and 8 ml of ice-cold water. The activity of HNMT was measured by the method described above.

Histamine release from the peripheral blood leukocytes of the AICU patients was examined using the method of Bae et al. (26). For the histamine release assay, 10 µg/ml of anti-human goat IgE antibody (KPL, Gaithersburg, MD, USA), which was determined as the optimal concentration in a preliminary experiment, was used. Histamine concentrations were measured using the Histamine EIA kit (Immunotech) according to the manufacturer’s instructions. The absorbance at 405 nm of each sample was measured in the Synergy HT microplate reader (BioTek Instruments). Histamine release is expressed as a percentage of total cellular histamine, based on the following formula: % histamine release = 100 × [stimulated histamine release – spontaneous histamine release]/(total cellular histamine release – spontaneous histamine release).

**Preparation of pHNMT CDS-3' UTR (939A or 939G) and in vitro functional study**

A 1382-bp fragment that included the coding sequence (CDS) plus the 3'-UTR of the human HNMT gene was prepared by PCR amplification with either the 939A homozygote or 939G homozygote human cDNA as template. The forward primer sequence was 5'-GAACCTTAAACCTTGTTCTG-3', and the reverse primer sequence was 5'-TTTGTGCTTCTCTTCTTCG-3'. The amplification conditions were: initial denaturation for 5 min at 95°C, followed by 34 cycles of 30 s at 95°C, 30 s at 50°C, and 80 s at 72°C, with a final 7 min of extension at 72°C. Each of the PCR products was cloned separately into pCR2.1-TOPO (Invitrogen), and then subcloned into the pcDNA3 vector (Invitrogen).

HMC-1 cells were transfected with the pHNMT CDS-3'-UTR constructs (1 µg) using the MP-100 microporator (Digital Biotech), as described above. The cell lysates of the transfected HMC-1 cells were collected after two washes with PBS (pH 7.2). The HNMT enzymatic activity of each cell lysate was measured using a previously described method (25). Briefly, 25.9 µl of cell lysate was incubated for 60 min at 37°C with 12.5 µmol histamine and 375 µmol [methyl-3H] Ado-Met (100 µM, 1 M Ci/mol; Amersham Pharmacia Biotech, Buckinghamshire, UK) in 100 nM potassium phosphate buffer (pH 7.5) in a total volume of 100 µl. Blanks were the samples to which no histamine was added. Radioactivity was measured in a Beckman LS-3801 liquid scintillation counter (Beckman Instruments, Irvine, CA, USA). The enzyme activity was measured as the formation of N'-methylhistamine per hour of incubation at 37°C. The enzyme activity was normalized to the total protein level.

After transfection of pHNMT CDS-3'-UTR constructs into HMC-1 cells, the cells were incubated for an additional 24 h, and then stimulated with 3 µM of calcium ionophore A23187 (Sigma) for 30 min. Histamine release was measured using the Histamine EIA kit (Immunotech, Marseille, France), according to the manufacturer's instructions. The absorption of each sample at 405 nm was recorded in the Synergy HT microplate reader (BioTek Instruments).

**In vivo functional study of the HNMT 939A > G SNP**

The HNMT enzymatic activities in the red blood cells (RBCs) of AICU patients and NC subjects were examined using the method of Pazmino and Weinhilboum (25). Briefly, 5-ml samples of blood were obtained from each subject and centrifuged at 800 g for 1 min at 4°C. The pellet was added to 3 ml of 0.9% NaCl and the suspension was recentrifuged at 800 g for 1 min at 4°C. The supernatant and buffy coat layers were then removed. The RBC pellet was washed twice with PBS and centrifuged at 800 g for 1 min at 4°C. The RBC pellet was resuspended in 2 ml of PBS and 8 ml of ice-cold water. The activity of HNMT was measured by the method described above.

Histamine release from the peripheral blood leukocytes of the AICU patients was examined using the method of Bae et al. (26). For the histamine release assay, 10 µg/ml of anti-human goat IgE antibody (KPL, Gaithersburg, MD, USA), which was determined as the optimal concentration in a preliminary experiment, was used. Histamine concentrations were measured using the Histamine EIA kit (Immunotech) according to the manufacturer’s instructions. The absorbance at 405 nm of each sample was measured in the Synergy HT microplate reader (BioTek Instruments). Histamine release is expressed as a percentage of total cellular histamine, based on the following formula: % histamine release = 100 × [stimulated histamine release – spontaneous histamine release]/(total cellular histamine release – spontaneous histamine release).

**Statistical analysis**

Statistical analyses were completed using the SPSS version 11 software (SPSS Inc., Chicago, IL, USA), and a P-value < 0.05 was considered to be significant. Significant departures of the genotype frequency from the Hardy–Weinberg equilibrium (HWE) at each polymorphic site were tested by chi-squared analysis and bonferroni method. Logistic regression models were used for the analysis of SNPs, while controlling for age and sex as covariates, using three alternative models (co-dominant, dominant, and recessive models). Differences in HNMT mRNA stability and protein expression levels between the two different genotypes were examined using the Repeated Measures (RM) ANOVA test. Differences in HNMT enzymatic activity and histamine release between the two different genotype groups were examined using the Mann–Whitney U-test.
Results
Clinical characteristics of chronic urticaria patients with aspirin hypersensitivity
The demographic features of the study subjects are summarized in Table 1. Significant differences were observed for age, atopy, and serum total IgE levels among the three groups ($P < 0.05$). Aspirin intolerant chronic urticaria patients showed a higher prevalence of atopy and higher total IgE levels than did ATCU patients ($P < 0.05$). No significant differences were noted for the prevalence of serum autoantibodies, which included anti-thyroglobulin, anti-microsomal, and anti-nuclear antibodies, between the AICU and ATCU groups ($P > 0.05$).

Case–control association study of HNMT polymorphisms
We investigated the genotype distributions of two common $HNMT$ SNPs, 314C>T (T105I) and 939A>G, among the three groups (Table 2). The genotype distributions of genetic polymorphisms did not deviate from the HWE ($P > 0.05$). The genotype frequency of the 939A>G polymorphism was significantly lower in the AICU group than in the NC group ($P = 0.024$, OR = 0.577, 95% CI = 0.334–0.823). The 939G allele frequency was also lower in the AICU group than in the NC group ($P = 0.030$, OR = 0.660, 95% CI = 0.443–0.982). The multiple comparison of 939A>G polymorphism was still significant when compared between AICU vs NC ($P_c = 0.048$). For the 314C>T SNP, no significant differences in the genotype and allele frequencies were detected among the three groups. As these two genetic polymorphisms of $HNMT$ were not in linkage disequilibrium ($|D'| = 0.74$ and $r^2 = 0.01$), the haplotype frequencies were not investigated. No significant correlations between urticaria severity scores and HNMT gene polymorphisms were noted for individual patients (data not shown).

Effects of the 939A>G polymorphism on HNMT mRNA stability
We examined the mRNA stability associated with the 939A>G polymorphism because of its location in the 3’-UTR. A comparison of the mRNA stabilities of the 939A and 939G alleles was performed by real-time

---

Table 1. Clinical characteristics of the study subjects

<table>
<thead>
<tr>
<th></th>
<th>AICU ($n = 111$)</th>
<th>ATCU ($n = 154$)</th>
<th>NC ($n = 152$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (positive/total)</td>
<td>53/111 (47.7%)</td>
<td>73/154 (47.4%)</td>
<td>67/152 (44.1%)</td>
</tr>
<tr>
<td>Age (years)*</td>
<td>35.41 ± 11.65</td>
<td>39.33 ± 11.71</td>
<td>30.86 ± 10.62</td>
</tr>
<tr>
<td>Atopy (presence/total)</td>
<td>79/106 (74.5%)</td>
<td>71/146 (48.6%)</td>
<td>15/138 (10.9%)</td>
</tr>
<tr>
<td>Log [serum total IgE (IU/ml)]*</td>
<td>2.24 ± 0.48</td>
<td>2.05 ± 0.47</td>
<td>1.55 ± 0.57</td>
</tr>
<tr>
<td>Anti-thyroglobulin antibody (positive/total)</td>
<td>11/67 (16.4%)</td>
<td>21/133 (15.8%)</td>
<td>–</td>
</tr>
<tr>
<td>Anti-microsomal antibody (positive/total)</td>
<td>7/67 (10.4%)</td>
<td>17/133 (12.8%)</td>
<td>–</td>
</tr>
<tr>
<td>Antinuclear antibody (positive/total)</td>
<td>14/70 (20.0%)</td>
<td>22/125 (17.6%)</td>
<td>2/66 (3.0%)</td>
</tr>
</tbody>
</table>

AICU, aspirin-intolerant chronic urticaria; ATCU, aspirin-tolerant chronic urticaria; NC, normal control; NA, not applicable. Bold character indicates significant $P$-value.

*This value is presented as the mean ± SD.

Table 2. Allele and genotype frequencies of the HNMT genetic polymorphisms

<table>
<thead>
<tr>
<th></th>
<th>AICU ($n = 111$)</th>
<th>ATCU ($n = 154$)</th>
<th>NC ($n = 152$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>314 C&gt;T (T105I)*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>102 (91.89%)</td>
<td>141 (91.56%)</td>
<td>138 (90.79%)</td>
</tr>
<tr>
<td>CT</td>
<td>9 (8.11%)</td>
<td>13 (8.44%)</td>
<td>13 (8.55%)</td>
</tr>
<tr>
<td>TT</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1 (0.66%)</td>
</tr>
<tr>
<td>$q$</td>
<td>0.041</td>
<td>0.042</td>
<td>0.049</td>
</tr>
<tr>
<td>939 A&gt;G</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>69 (62.16%)</td>
<td>82 (53.25%)</td>
<td>74 (48.88%)</td>
</tr>
<tr>
<td>AG</td>
<td>34 (30.83%)</td>
<td>62 (40.26%)</td>
<td>63 (41.45%)</td>
</tr>
<tr>
<td>GG</td>
<td>8 (7.21%)</td>
<td>10 (6.49%)</td>
<td>15 (9.87%)</td>
</tr>
<tr>
<td>$q$</td>
<td>0.225</td>
<td>0.266</td>
<td>0.306</td>
</tr>
</tbody>
</table>

314 C>T (T105I)*

$|D'| = 0.74$, $r^2 = 0.01$.

HNMT, histamine N-methyltransferase; AICU, aspirin-intolerant chronic urticaria; ATCU, aspirin-tolerant chronic urticaria; NC, normal control; NA, not applicable. Each $P$-value was calculated using codominant, dominant, and recessive models. Logistic regression analysis was used to control for age and sex as covariates. Bold character indicates significant $P$-value.

*T, threonine; I, isoleucine. $q$, minor allele frequency.
PCR for HMC-1 cells that were transfected with each genotype (939A or 939G) in the pEGFP-HNMT 3’-UTR construct (Fig. 1A). The mRNA level of the 939G-fused reporter gene was significantly more stable than that of the 939A-fused reporter gene ($P < 0.001$; Fig. 1B). The mRNA level of the 939A-fused reporter gene decreased dramatically in a time-dependent manner after treatment with actinomycin D, whereas the mRNA level of the 939G-fused reporter gene decreased slightly (Fig. 1B). A comparison of the EGFP protein expression profiles related to the 939A > G polymorphism was performed by measuring the fluorescence signals from transfected HMC-1 cells. The fluorescence intensity of the 939G-fused reporter gene was significantly higher than that of the 939A-fused reporter gene ($P = 0.048$; Fig. 1C). The modulation of mRNA stability associated with the 939A > G polymorphism was replicated in other cell lines [human lung epithelial cells (A549) and human leukemic monocyte lymphoma cells (U937); data not shown].

Effects of the 939A>G polymorphism on HNMT enzymatic activity and histamine release

We examined the contribution of the HNMT gene to the enzymatic activity of HNMT and histamine release both in vitro and in vivo. First, using the pHNMT CDS-3’-UTR construct (Fig. 2A), we determined whether the HNMT 939A > G polymorphism altered the enzymatic activity of HNMT. After transient transfection of the pHNMT CDS-3’-UTR construct into HMC-1 cells, the cells transfected with the HNMT 939G construct showed significantly higher HNMT enzymatic activities than those transfected with the HNMT 939A construct ($P < 0.001$; Fig. 2B). Histamine release from the transfected HMC-1 cells differed significantly between the HNMT 939A and HNMT 939G constructs. The HNMT 939G construct showed significantly lower histamine content than the HNMT 939A construct ($P < 0.001$; Fig. 2C).

Secondly, we compared the enzymatic activities of HNMT and the levels of basophil histamine release related to the HNMT 939A > G polymorphism using RBC lysates and basophils from the AICU patients. The AICU patients who had the 939AG or 939GG genotype showed significantly higher enzymatic activities in their RBCs than did patients who had the 939AA genotype ($P = 0.007$; Fig. 3A). The average level of enzymatic activity was significantly different between the 939AA genotype and the 939AG or 939GG genotype (177.81 ± 57.13 vs 348.87 ± 321.90, $P = 0.007$; Table 3) in the

**Figure 1.** Effect of the HNMT 939A > G polymorphism on mRNA stability and protein expression. mRNA levels of the pEGFP-HNMT 3’UTR reporter construct in HMC-1 cell were assessed by real-time PCR after actinomycin D (10 μg/ml) treatment. (A) Schematic representation of EGFP reporter gene constructs including the HNMT 3’UTR region with the 939A > G polymorphism. (B) EGFP mRNA stability according to the 939A > G polymorphism. (C) EGFP protein expression according to the 939A > G polymorphism. Each experiment was conducted three different times for each sample. RFU, relative fluorescence unit. The $P$-value was determined by a RM ANOVA test.
Figure 2. *In vitro* functional study of HNMT 939A>G polymorphism. (A) Schematic representation of the HNMT gene construct containing the 939A>G polymorphism. (B) HNMT enzyme activity was examined by measuring the formation of \( \text{N}_{\text{S}} \)-methylhistamine in pHNMT CDS-3’UTR-transfected HMC-1 cells. The relative enzyme activity (CPM, count per minute) is represented as the ratio of the activity to the enzyme activity in the cells transfected with the empty control vector pcDNA3. (C) Histamine releasing was examined by measuring the histamine release stimulated by calcium ionophore A2319 (3 \( \mu \)M) in pHNMT CDS-3’UTR-transfected HMC-1. Each experiment was conducted in triplicate and the results are expressed as the mean ± SD for three independent experiments (\( n = 9 \)). The \( p \) value was determined by a Mann–Whitney test.

Figure 3. *In vivo* functional study of HNMT 939A>G polymorphism. (A) HNMT enzyme activity was examined by measuring the formation of \( \text{N}_{\text{S}} \)-methylhistamine in Human RBC lysate. (B) Histamine releasing from peripheral basophils after stimulation with anti-IgE (10 \( \mu \)g/ml) in AICU patients (\( n = 23 \)). –This value is presented the mean value. The \( P \)-value was determined by a Mann–Whitney test.
**Association of HNMT genetic polymorphism with AICU**

<table>
<thead>
<tr>
<th></th>
<th>AICU</th>
<th>NC</th>
<th>( p )-value</th>
<th>AICU</th>
<th>NC</th>
<th>( p )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzyme activity*</td>
<td>164.47 (147.94–205.32)</td>
<td>235.12 (176.10–338.63)</td>
<td>0.021</td>
<td>457.22 (327.83–930.52)</td>
<td>476.49 (338.55–848.86)</td>
<td>0.564</td>
</tr>
<tr>
<td>Histamine release*</td>
<td>100.00 (44.70–123.21)</td>
<td>22.21 (6.72–56.42)</td>
<td>0.012</td>
<td>35.06 (23.73–103.93)</td>
<td>65.81 (62.44–97.78)</td>
<td>0.713</td>
</tr>
</tbody>
</table>

HNMT, histamine \(N\)-methyltransferase; AICU, aspirin-intolerant chronic urticaria; NC, normal control.

*This value is presented as the median (25th, 75th) of subjects.

<table>
<thead>
<tr>
<th></th>
<th>AA</th>
<th>AG + GG</th>
<th>( p )-value</th>
<th>AA</th>
<th>AG + GG</th>
<th>( p )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzyme activity*</td>
<td></td>
<td></td>
<td></td>
<td>0.021</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histamine release*</td>
<td></td>
<td></td>
<td></td>
<td>0.012</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


AICU patients, whereas no such differences were noted in the RBCs of the NC subjects (Table 3). Moreover, there was no significant difference in HNMT enzymatic activity associated with the 314C > T polymorphism between the AICU patients and NC subjects (data not shown).

Moreover, AICU patients carrying the 939A allele showed significantly higher histamine release from peripheral blood basophils than did those carrying the 939G allele \((P = 0.012; \text{Fig. 3B})\). There was no difference in basophil histamine release associated with the 314T>C polymorphism between the AICU patients and NC subjects (data not shown).

**Discussion**

Aspirin and other nonsteroidal anti-inflammatory drugs have been reported to aggravate disease symptoms in 20–30% of patients with chronic urticaria (14), although the pathogenic mechanism underlying AICU remains unclear. Previously, we reported a prevalence of aspirin hypersensitivity of 35.7% in chronic urticaria patients in a Korean population (15). Aspirin intolerant chronic urticaria patients showed higher frequencies of atopic status and higher total IgE levels compared to ATCU patients (15). According to our previous report on the genetic mechanism of AICU (26), histamine release appears to be modulated by genetic polymorphism of the FCER1\(\alpha\) gene, especially in AICU patients, and acts as an important mediator in the pathogenesis of AICU.

In our preliminary experiment, we found that AICU patients had lower levels of HNMT enzymatic activity and higher total cellular histamine levels, as compared to NC. Therefore, we hypothesized that the histamine metabolizing enzyme, HNMT, plays an important role in the pathogenesis of AICU by modulating the histamine level, and we performed a genetic association study of two common genetic polymorphisms of the HNMT gene.

The nonsynonymous 314C > T (T105I) SNP of HNMT has been reported to be associated with asthma (18), and the 314T (105I) allele has been linked to lower levels of HNMT protein expression and enzymatic activity than the 314C (105T) allele (17, 27). In this study, we found no significant genetic association between HNMT 314C > T polymorphism and chronic urticaria. The minor allele frequency \((q)\) of this polymorphism was close to 0.05 in a Korean population, which is similar to the values obtained for other Asian populations including Chinese \([q = 0.06, (28)]\) and Japanese \([q = 0.05, (29)]\), but lower than that obtained for American Caucasians \([q = 0.09, (16)]\).

As the HNMT 939A > G polymorphism is located on the regulatory 3'-UTR, it has been reported to be related to HNMT enzymatic activity (20). In a normal Chinese population, the HNMT activity in RBCs for the 939GG genotype has been found to be higher than that for the 939AG or 939AA genotype (20). However, there are no published data to suggest a significant association of this polymorphism with any allergic disease. In this study, we demonstrate a significant association between the HNMT 939A > G polymorphism and the AICU phenotype, and provide supporting evidence from in vitro and in vivo functional studies. The AICU patients showed a significantly lower frequency of the variant genotype of this polymorphism than did the NC subjects, which suggests that the 939A allele contributes to the development of AICU.

Previous biochemical and genetic studies have demonstrated that individual variation in the HNMT activity in RBCs is regulated by inheritance (30, 31), and that HNMT activity can be modulated by the HNMT 939A > G polymorphism (20). We examined the functional variability in relation to the 939A > G polymorphism both in vitro and in vivo, and found that the variant genotype of the 939A > G polymorphism showed higher mRNA stability and increased enzymatic activity. Variability of the HNMT enzyme activity related to the 939A > G polymorphism was confirmed for the RBCs of the AICU patients but not for the RBCs of the NC subjects. Moreover, basophil histamine release was found to be significantly different with respect to the 939A > G polymorphism for the AICU patients but not for the NC subjects. These data suggest that the SNP at the 3'-UTR (939A > G) modulates mRNA stability, alters HNMT enzymatic activity, and affects the cellular histamine levels in AICU patients, and may have some effects in other type of chronic urticaria patients carrying this gene polymorphism.

According to our previous report, variable histamine release as a result of genetic polymorphism, e.g. the FCER1\(\alpha\) SNP at –344C > T, is a very important event in the pathogenesis of AICU (26). In this study, we also
found that the cellular histamine level was modulated by \textit{HNMT} polymorphism (939A > G), and that this effect was significant in AICU patients but not in NC subjects. Therefore, we propose that the genetic contribution of \textit{HNMT} polymorphism to the cellular histamine level is a significant molecular genetic mechanism of AICU.

The present study has a few potential limitations. We focused on only two common genetic polymorphisms of \textit{HNMT}, so we cannot exclude the possibility that the 939A > G SNP may interact with other polymorphisms. Therefore, further studies are needed to investigate other regulatory regions of \textit{HNMT}. Moreover, the level of bioactive histamine can be regulated by the synthesizing enzyme HDC. Therefore, further studies are necessary to investigate the genetic contribution of the \textit{HDC} gene to AICU.

In conclusion, the 939A > G polymorphism of the \textit{HNMT} gene may decrease HNMT enzyme activity by decreasing mRNA stability, thereby increasing the cellular histamine level, which eventually leads to the development of AICU.

\textbf{Acknowledgments}

This study was supported by a grant from the Korea Health 21 R&D Project of the Ministry of Health & Welfare, Republic of Korea (03-PJ10-PG13-GD01-0002 and A050571).

\textbf{References}


